

DEV Day 3 Interpretation Questions (and Answers)

1. Interpret your data from observing the zebrafish embryos. This should include the answers to the questions:
 - a. What stage embryos did you observe?
They should have seen segmentation through hatching on Dev 3.
 - b. What did you observe in these embryos that was expected and unexpected?
They should have seen somites, eyes, brains, hearts, circulation, etc.
 - c. Did you observe differences between untreated and LiCl treated embryos? If so, what differences?
The biggest "phenotype" was that most of the LiCl treated embryos died! On TR, people may have noticed that there was no pigmentation, no eyes, and maybe also that the tails seem to be a bit shorter in the treated fish. The WF section did not get to see LiCl treated embryos.
 - d. Based on what you know about teratogens, would you expect to see any differences between untreated and LiCl treated embryos if the treatment had been done at 24 hours post fertilization?
Fang said that teratogens affect either mRNA or proteins, and these changes result in developmental changes in the embryo. At 24 hours post fertilization the zebrafish embryo is nearly fully formed and therefore should not show any signs of change due to the LiCl treatment.

2. Interpret data from observing your gel. This should include the answers to the questions:
 - a. Did you see bands in every lane of the gel you loaded?
 - b. What were the amounts of rRNA in each of stages 1-4? (Were they equal?)

Answers will vary, but hopefully they saw equal amounts of RNA in each of their lanes. They may have seen bands in their control lanes. If not, it might be because they loaded so LITTLE an amount.

- c. Why can you see a band in the lane of your agarose gel that is supposed to be our negative control?

In the lane with the negative control for the Northern, they SHOULD see the DNA! This is because EtBr stains all nucleic acids nonspecifically. It is during the NORTHERN blot that we will be using the pBSK as a negative control. Since pBSK does not have any sequences complementary to our zcyt1 probe, the probe will not bind, and no band will be observed after development.

DEV Day 3 Interpretation Questions (and Answers) (continued)

3. Instead of using the protocols at listed, you decide to be creative when running random hex-priming labelling. Explain what might happen in the following scenarios:
- a. Instead of using 0.65 mM dTTP + 0.35 mM dig-11-dUTP, you feel like kicking it up a notch by using **1.0 mM dig-11-dUTP!**

If we only used dig-11-dUTP, the DNA polymerase would always incorporate dig-11-dUTP when the template DNA has an A nucleotide. Dig-11-dUTP, however, is an extremely large molecule, in contrast to dTTP and dUTP. This becomes a problem when trying to synthesize DNA from a template with multiple A nucleotides in a row; you sterically cannot put more than one Dig-11-dUTP in a row! Thus, by using only dig-11-dUTP, our template would not be labeled as effectively—and you'd see a less strong signal on the Northern blot.

- b. You have run out of the Klenow DNA Polymerase I, and instead of ordering some more, you decide to use left over Taq Polymerase from your RDM module.

Taq polymerase is less active at the lower temperature of 37 C. It is optimally active at much higher temperatures—remember that we used an extension temperature of 72 C for PCR in RDM! If polymerase is not as active at this temperature, our probe labelling would be less successful because the primers would not be extended efficiently. This would result in a weaker signal on the Northern blot.